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(54) Title: PHARMACEUTICAL WITH IMMUNOMODULATING ACTIVITY

(57) Abstract

Pharmaceutical peptide preparations for inducing a heightened state of anti-microbial cellular or humoral immunity in a subject in need thereof consisting essentially of an L-Lys-L-Glu or L-Glu-L-Trp preparation and a pharmaceutically acceptable carrier.

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PHARMACEUTICAL WITH IMMUNOMODULATING ACTIVITY

Field of the Invention

The invention relates to peptides useful as pharmaceutical agents having immunomodulatory activity and particularly to methods of using therapeutic dipeptides L-lysyl-L-glutamic acid (L-Lys-L-Glu) and L-glutamyl-L-tryptophane (L-Glu-L-Trp).

Background of the Invention

Tissue extracts that affect proliferation and/or differentiation of T-lymphocytes have been reported, including thymus extracts of animal origin, e.g., "thymosine" (1), "thymaline" (2), "T-activine" (3), "thymosin-α_1 (4) and others. Commonly tissue extracts exist as complex mixtures that includes polypeptides. Widespread use of such biological mixtures in medical practice has been hindered by the lack of highly purified and characterized sources, as well as by the low yields obtainable, and, once isolated, the considerable variability of chemical and physical properties potentially effecting potency, stability, toxicity, and safety.

"Thymogen" (5) is reportedly prepared as a synthetic L-Glu-L-Trp product whose design is in accord with a dipeptide in a fraction isolated a thymalin (2) extract of bovine thymus.

T-lymphocytes participate in cellular and humoral immune responses to foreign antigens that are triggered following binding of ligand to cell surface receptors. The CD2 receptor on T-lymphocytes, (previously known as the erythrocyte rosette receptor-abbreviated E-receptor) serves a dual role as both an adhesion molecule and a signal transducing molecule. CD2 binding to LFA-3 may promote adhesive binding of T-lymphocytes to $LFA3^+$ (CD58) B-lymphocytes and thymic epithelial cells. CD2 binding to CD59 and CD48 ligands may facilitate binding to other cells. CD2 is a cell surface molecule bound by PHA and involved in PHA-induced lymphocyte blastogenesis. Binding of anti-CD2 antibodies to the CD2 receptor is capable of triggering T-lymphocyte blastogenesis that may be independent of the CD3/T cell receptor complex. CD4 and CD8 define MHC class specificity of T helper and cytotoxic T lymphocytes, respectively. (Paul, W.E. Ed. 1993. "Fundamental Immunology, 3rd. Edition", Raven Press, N.Y. pp.541-5.)

Summary of the Invention

Disclosed herein are the results of studies showing that treatments with L-Lys-L-Glu dipeptide and Thymogen (L-Glu-L-Trp) are capable of increasing expression of CD2 on T-lymphocytes and thymocytes, altering tissue distribution of lymphocyte

subpopulations in experimental animal model systems, and stimulating immune cells involved in antimicrobial cellular immunity. In *in vitro* studies L-Lys-L-Glu and Thymogen also stimulated increased expression of CD4 (but not CD8) on peripheral blood lymphocytes isolated from patients with secondary immunodeficiency syndromes. In experimental animal model systems, treatment with L-Lys-L-Glu and Thymogen altered the tissue distribution of T-lymphocytes and lymphocytes bearing cell surface Fc-receptors, and L-L, and L-Lys-L-Glu increased splenic weight and T-lymphocyte content. When delivered locally (i.e., intraperitoneally), the subject L-Lys-L-Glu and Thymogen treatments increased the activation state of resident macrophages in experimental animal studies (as measured by NBT reduction); and, promoted neutrophil infiltration in response to a sterile inflammatory mediator (proteose peptone). The combined results show that L-Lys-L-Glu and Thymogen treatments stimulated immune cells and reticuloendothelial tissues participating in antimicrobrial mechanisms of cellular and humoral immunity, and indicate that the subject treatments heighten the state of antimicrobial immunity. For these and other reasons (disclosed herein) L-Lys-L-Glu and Thymogen are termed "immunomodulators" capable of heightening the state anti-microbial cellular or humoral immunity in a treated subject.

Embodiments of the invention provide methods of treatment with L-Lys-L-Glu (KE) and Thymogen to induce a heightened state of anti-microbial cellular or humoral immunity in a subject in need thereof.

Detailed Description of the Preferred Embodiment

While conducting comparative *in vitro* dose-response studies of different synthetic peptides and Thymogen (L-Glu-L-Trp), the L-Lys-L-Glu dipeptide was discovered to upregulate E-rosette formation by T-lymphocytes. Subsequent *in vitro* and *in vivo* model studies revealed new therapeutic effects of both dipeptides on the reticuloendothelial system.

The results of *in vitro* studies disclosed in EXAMPLES 2-4, below, show that dipeptide L-Lys-L-Glu and Thymogen increased expression of accessory molecules on the surface of thymocytes and mature T-lymphocytes as evidenced by i) increased E-rosette forming cells (E-RFC) in thymocyte cultures after incubation with either dipeptide; ii) increased E-RFC in cultures of thymocytes from aged animals after incubation with either dipeptide; and, iii) increased expression of OKT 4^+ in cultures of human peripheral blood T-lymphocytes from patients with secondary immunodeficiency syndromes following incubation with either dipeptide. Increased expression of CD2 and CD4 accessory

molecules on T-lymphocytes (EXAMPLES 2-4) is compatible a heighten the state of innate or induced immunity to infection, e.g., by upregulating T-helper and cytotoxic T-lymphocytes to respond to lower levels of antigen. On a weight basis L-Lys-L-Glu appeared to be more than 100-times more effective than Thymogen at increasing cell surface expression of CD2 and CD4 on lymphocytes. Neither dipeptide upregulated CD8 expression on lymphocytes. *In vivo* studies, disclosed in EXAMPLES 5-10 (below), show immunological effects of both dipeptides in experimental animal models. (*In vivo* studies are discussed further, below, in regard to the treatment methods using L-Lys-L-Glu and L-Glu-L-Trp.)

As used herein the symbols for amino acids are according to the IUPAC-IUB recommendations published in Arch. Biochem. Biophys. 115: 1-12, 1966 with the following single letter symbols for the amino acids: namely,

L, Leu, Leucine	V, Val, Valine	Y, Tyr, Tyrosine	D, Asp, Aspartic Acid
I, Ileu, Isoleucine	P, Pro, Proline	W, Trp, Tryptophan	E, Glu, Glutamic Acid
M, Met, Methionine	G, Gly, Glycine	N, Asn, Asparagine	K, Lys, Lysine
T, Thr, Threonine	A, Ala, Alanine	Q, Gln, Glutamine	R, Arg, Arginine
F, Phe, Phenylalanine	S, Ser, Serine	C, Cys, Cysteine	H, His, Histidine

The symbols for protective groups used in peptide synthesis are described in Schnidex and Lebke, "The Peptides", Academic Press, N.Y. 1965, e.g., Boc, t-butyloxycarbonyl and Bzl, benzyl. Other abbreviations used are e.g., HPLC, high pressure liquid chromatography; TFA, trifluoroacetic acid; KD, dissociation constant; Ka, association constant; Keq, equilibrium constant; f.a., fatty acid; E, erythrocyte; E-RFC, E-rosette forming cell; EA, erythrocyte antibody; EAC, erythrocyte antibody complement; APC, antigen presenting cell; PHA, phytohemagglutinin; Con-A, concanavalin A; LPS, lipopolysaccharide; IL, interleukin; CSF, colony stimulating factor; IFN, interferon; CTL, cytotoxic T-lymphocyte; NK-cell, natural killer cell; BM, bone marrow; PBL, peripheral blood leukocyte; LN, lymph node; KLH, keyhole limpet hemocyanin; ELISA, enzyme linked immunosorbent assay; FIA, fluorescence immunoassay; TRF, time resolved florescence assay; and, RIA, radioimmunoassay.

The following terms are intended to have meanings as follows: namely,

"CD2" is intended to mean the lymphocyte cell surface accessory molecule that is the homo- and heterotypic receptor mediating binding of heterologous erythrocytes (E; e.g., rabbit or sheep erythrocytes) to lymphocytes to form E-rosettes (as disclosed in Paul, W.E. Ed. "Fundamental Immunology, 3rd. Edition, Raven Press, N.Y. at page 562; incorporated herein by reference). Lymphocytes having cell surface CD2, when capable